Haploinsufficiency for *AAGAB* causes clinically heterogeneous forms of punctate palmoplantar keratoderma

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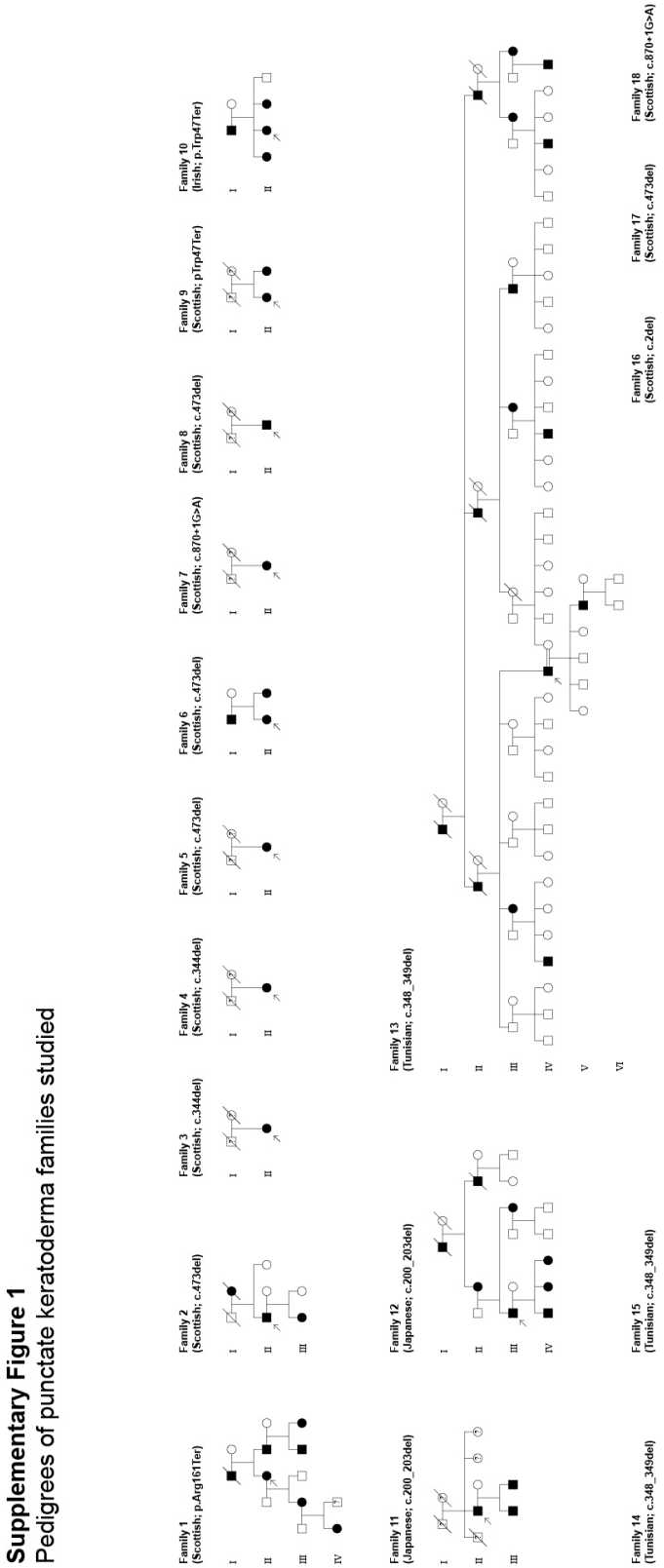
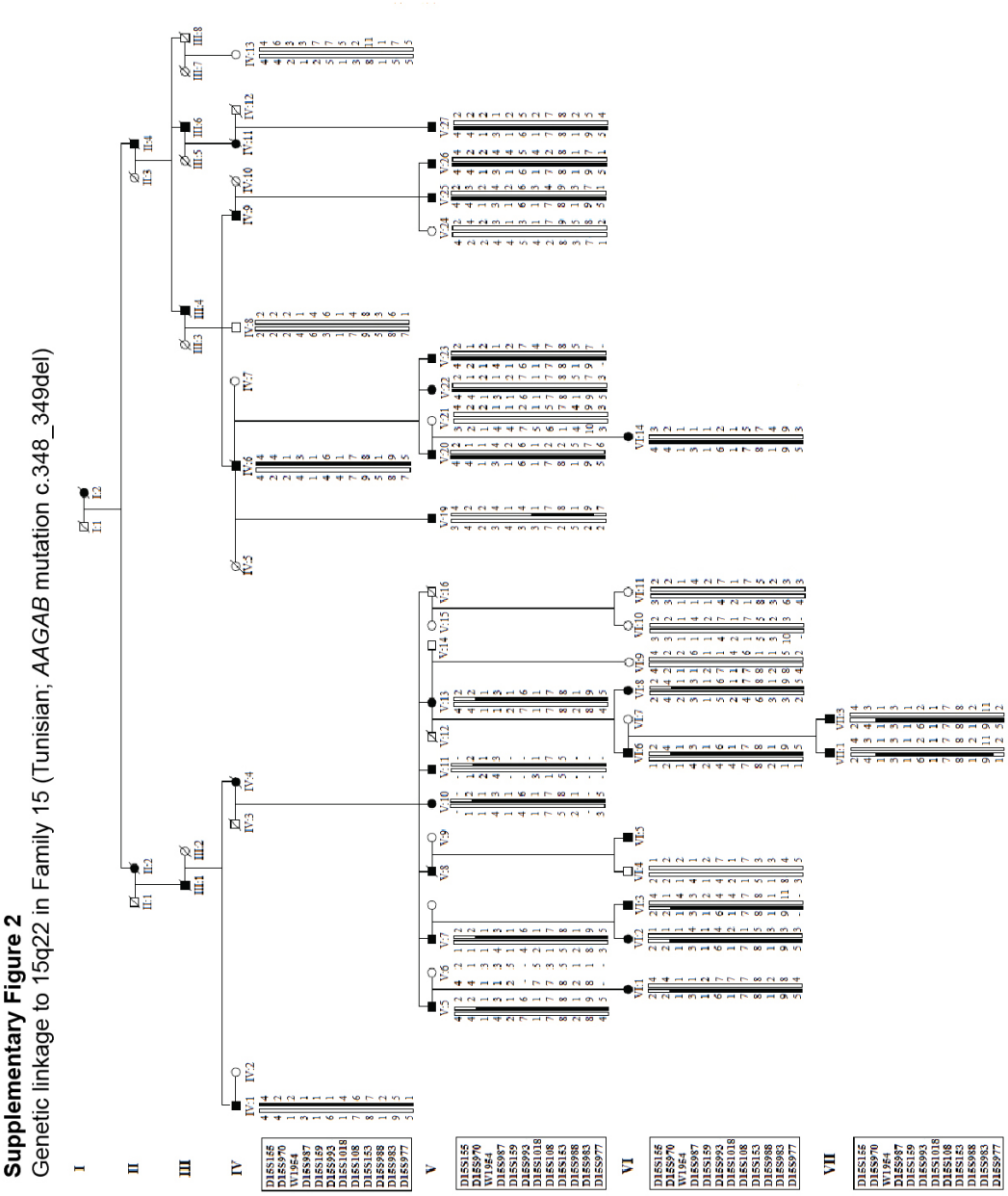
Supplementary Figure 1

Pedigrees of the 18 PPKP1 families studied. Arrow indicates proband. Ancestry and AAGAB mutations are annotated.



Supplementary Figure 2

Microsatellite linkage analysis on part of Family 15 (see **Supplementary Figure 1**). Disease haplotypes are shaded black. The maximum 2-point lod score of 8.18 at θ =0, was obtained with marker D15S983 (see **Supplementary Table 1**).



Supplementary Figure 3

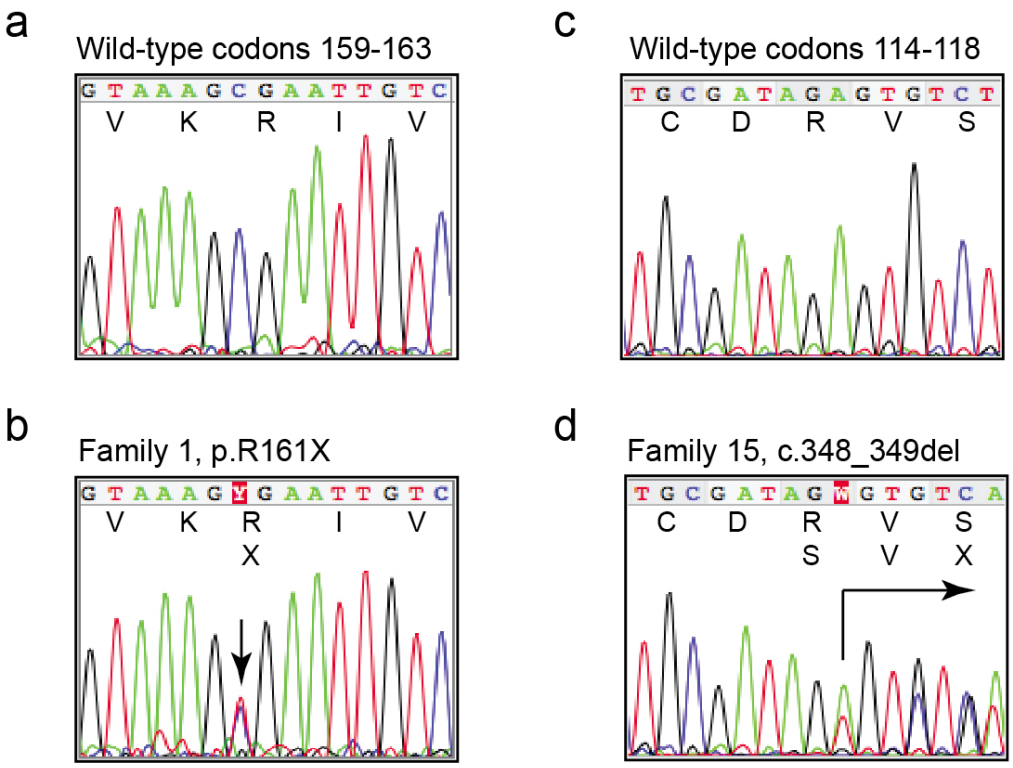
Examples of mutations identified in *AAGAB* in PPKP1 families

(a) Wild-type sequence from an unaffected control individual, spanning codons 159-163 of *AAGAB*.

(b) The same region of AAGAB shown in (a) derived from the proband in Family 1, showing heterozygous nonsense mutation c.148C>T (predicting protein alteration p.R161X).

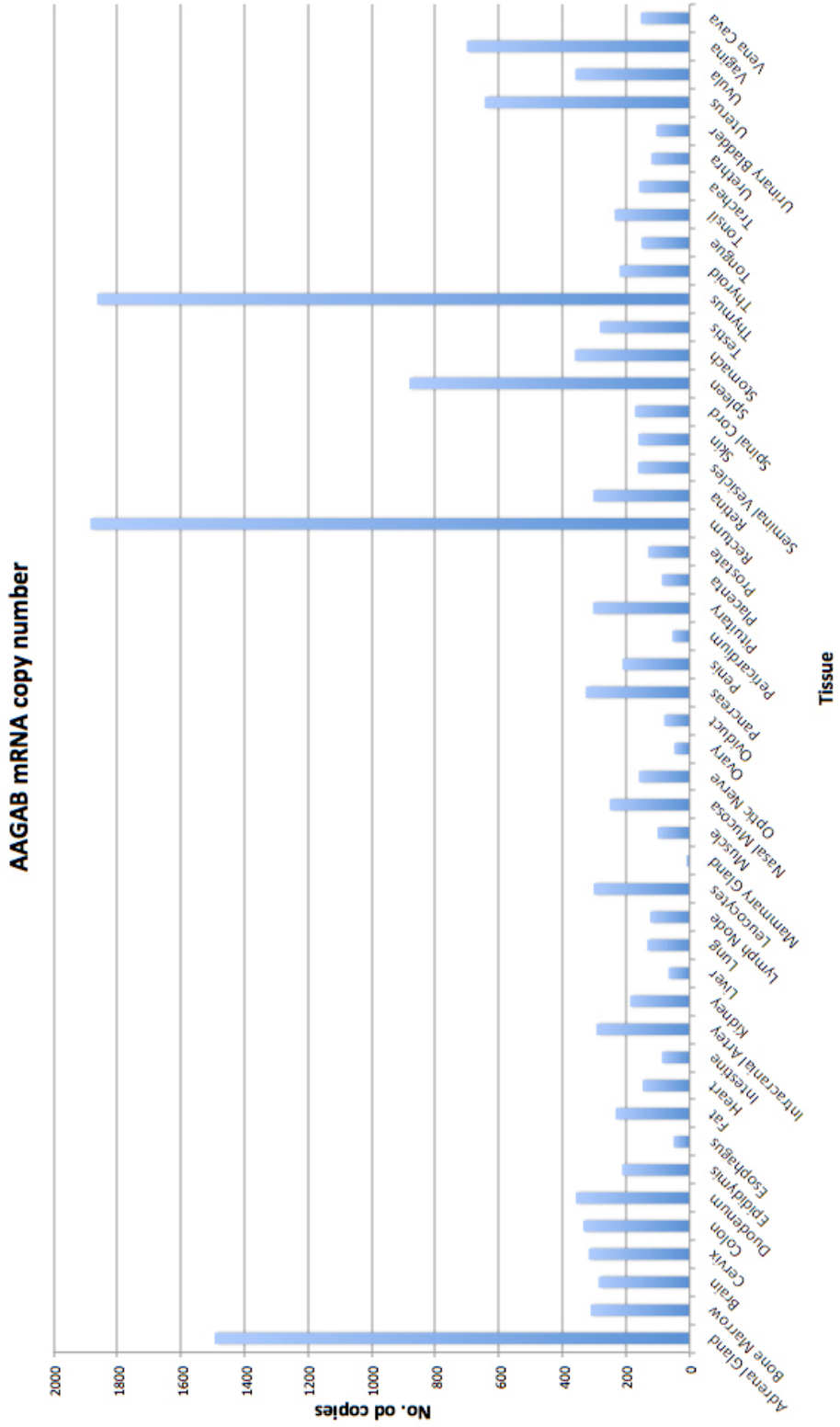
(c) Wild-type sequence from an unaffected control individual, spanning codons 114-118 of *AAGAB*.

(d) The same region of *AAGAB* shown in (c) derived from the proband in Family 15, showing heterozygous 2 bp deletion mutation c.348-349del (predicting protein alteration p.R116Sfs*1).



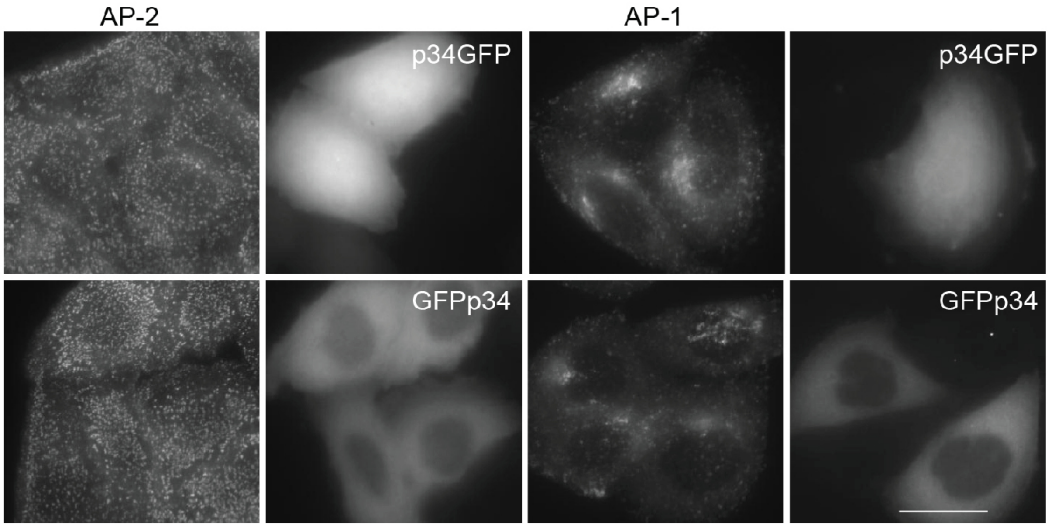
Supplementary Figure 4

Quantitative reverse-transcriptase PCR for *AAGAB* (p34) mRNA across a panel of normalized cDNAs from 48 different human tissues. Gene expression is expressed as copy number relative to a plasmid control. These data show that *AAGAB* is widely expressed, including in skin.



Supplementary Figure 5

HeLa cells were transiently transfected with either C-terminally (p34GFP) or N-terminally (GFPp34) GFP-tagged p34 constructs, and the localization of AP-1 and AP-2 visualized using immunofluorescence. Note that whether p34 is depleted or overexpressed there is no significant change in the localization of AP-1 and AP-2 adaptor complexes (see also **Figure 4**). Bar = 20 μm.



Supplementary Figure 6

Protein domain analysis of the p34 protein shows a Rab-like GTPase domain in addition to the adaptin-binding domain defined by yeast 2-hybrid analysis[10].

Human AAGAB p34: NCBI Conserved Domain analysis
(http://www.ncbi.nlm.nih.gov/cdd)

Protein sequence annotation

Accession NP_078942 length=315 derived from genomic assembly hg19

MAAGVPCALVTSCSSVFSGDQLVQHILGTEDLIVEVTSNDAVRFYPWTIDNKYYSADINLCVV

PNKFLVTAEIAESVQAFVVYFDSTQKSGLDSVSSWLPLAKAWLPEVMILVCDRVSEDGINRQK

AQEWCIKHGFELVELSPEELPEEDDDFPESTGVKRIVQALNANVWSNVVMKNDRNQGFSLLN

SLTGTNHSIGSADPCHPEQPHLPAADSTESLSDHRGGASNTTDAQVDSIVDPMLDLDIQELASL

TTGGGDVENFERLFSKLKEMKDKAATLPHEQRKVHAEKVAKAFWMAIGGDRDEIEGLSSDEE

H

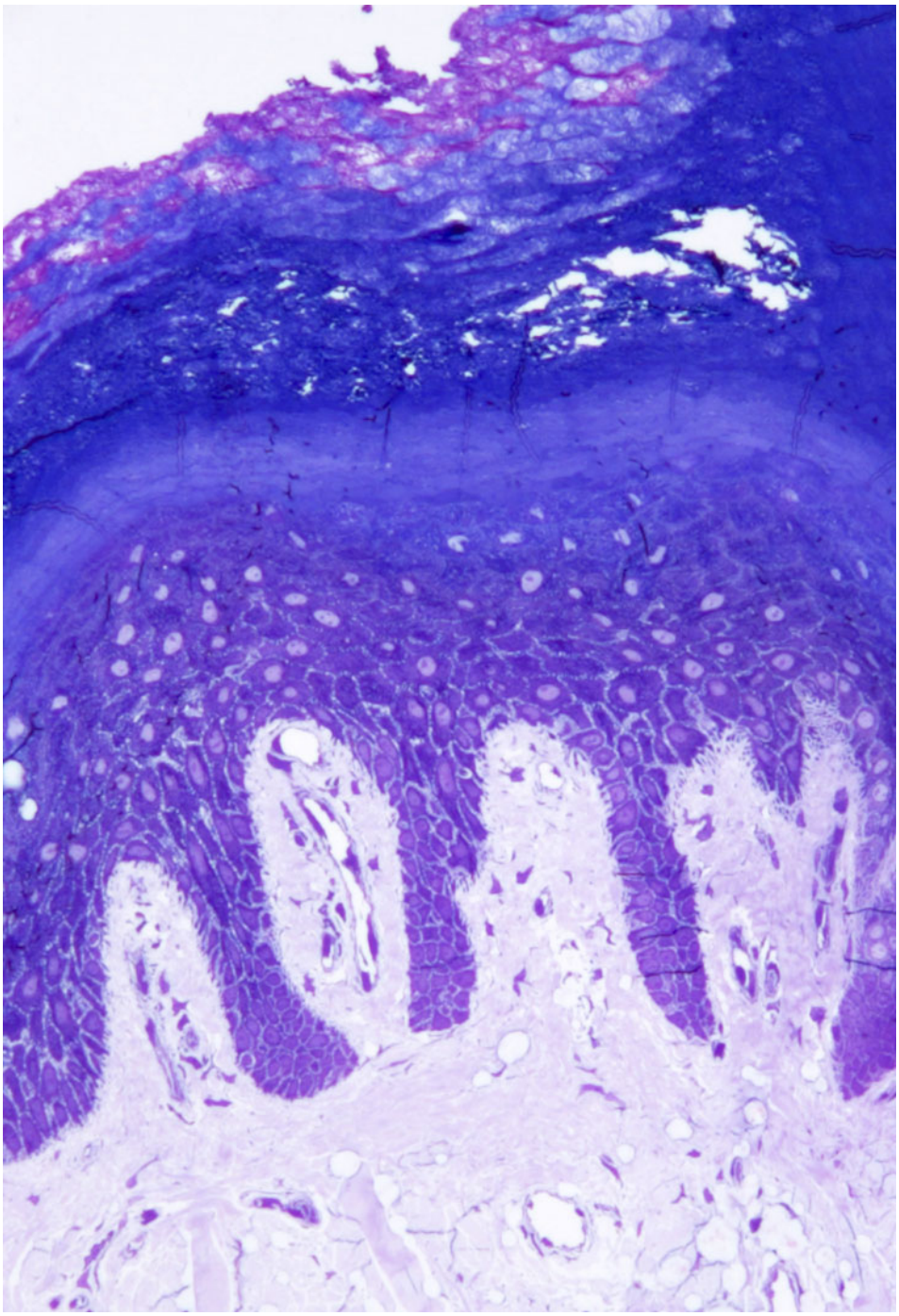
Domains:

Rab-like GTPase domain (P-loop_NTPase)
Adaptin-binding

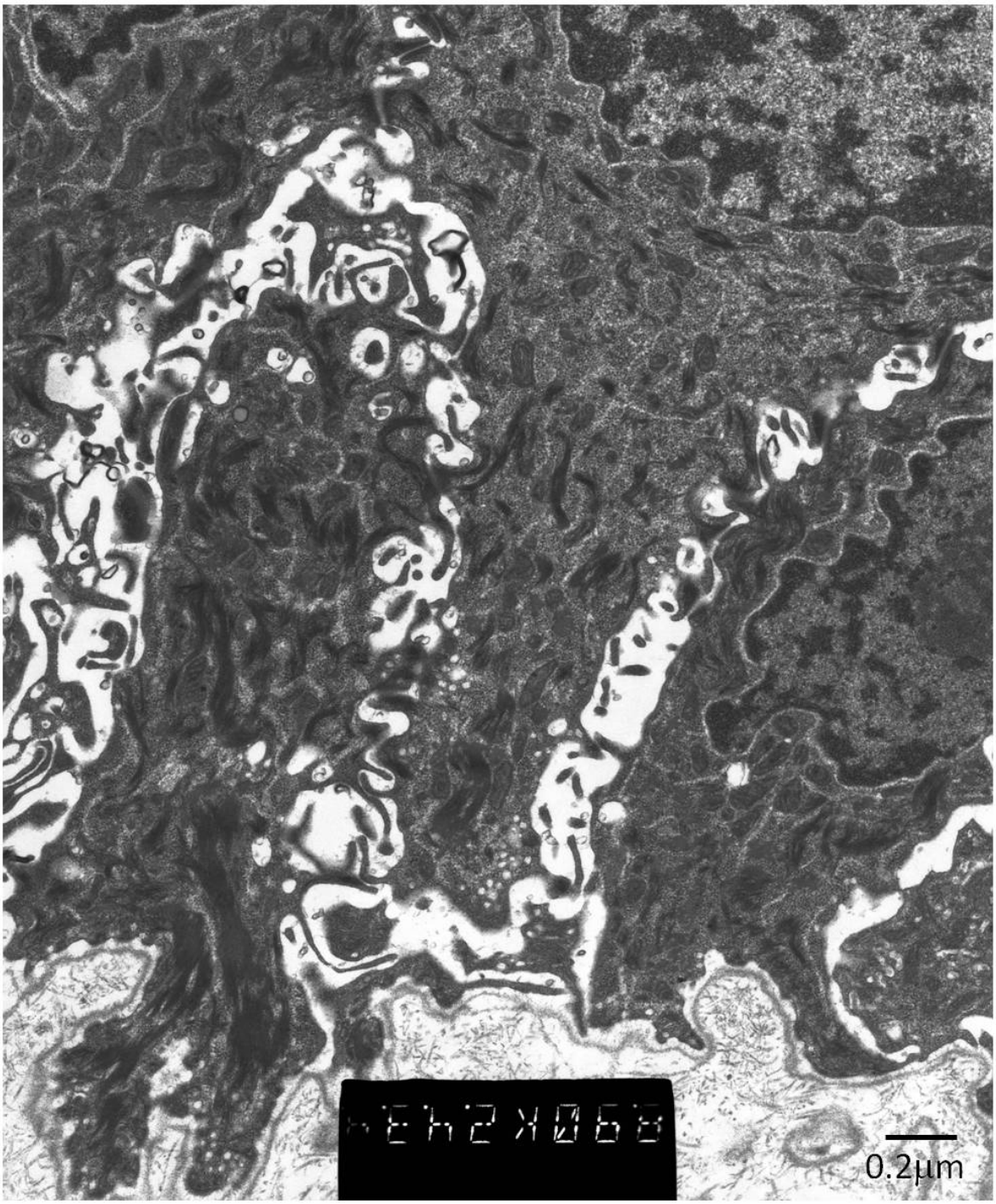
Description from NCBI Conserved Domains:

Ras-related in brain (Rab) family of small guanosine triphosphatases (GTPases)
Rab GTPases form the largest family within the Ras superfamily. There are at least 60 Rab genes in the human genome, and a number of Rab GTPases are conserved from yeast to humans. Rab GTPases are small, monomeric proteins that function as molecular switches to regulate vesicle trafficking pathways. The different Rab GTPases are localized to the cytosolic face of specific intracellular membranes, where they regulate distinct steps in membrane traffic pathways. In the GTP-bound form, Rab GTPases recruit specific sets of effector proteins onto membranes. Through their effectors, Rab GTPases regulate vesicle formation, actin- and tubulin-dependent vesicle movement, and membrane fusion. GTPase activating proteins (GAPs) interact with GTP-bound Rab and accelerate the hydrolysis of GTP to GDP. Guanine nucleotide exchange factors (GEFs) interact with GDP-bound Rabs to promote the formation of the GTP-bound state. Rabs are further regulated by guanine nucleotide dissociation inhibitors (GDIs), which mask C-terminal lipid binding and promote cytosolic localization. While most unicellular organisms possess 5-20 Rab members, several have been found to possess 60 or more Rabs; for many of these Rab isoforms, homologous proteins are not found in other organisms. Most Rab GTPases contain a lipid modification site at the C-terminus, with sequence motifs CC, CXC, or CCX. Lipid binding is essential for membrane attachment, a key feature of most Rab proteins. Since crystal structures often lack C-terminal residues, the lipid modification site is not available for annotation in many of the CDs in the hierarchy, but is included where possible.

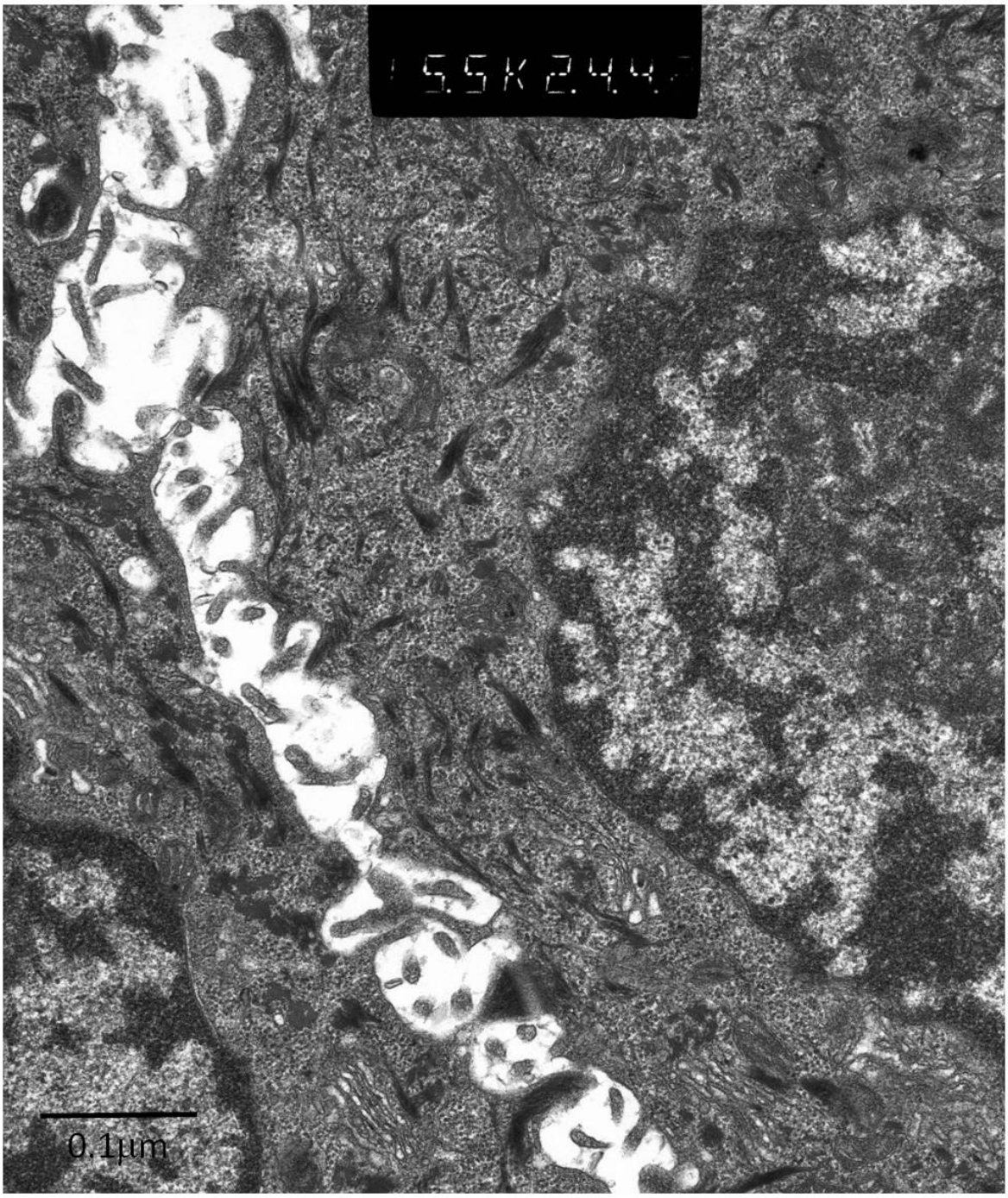
Supplementary Figure 7
Additional electron micrographs showing defects in vesicle populations in lesional epidermis.



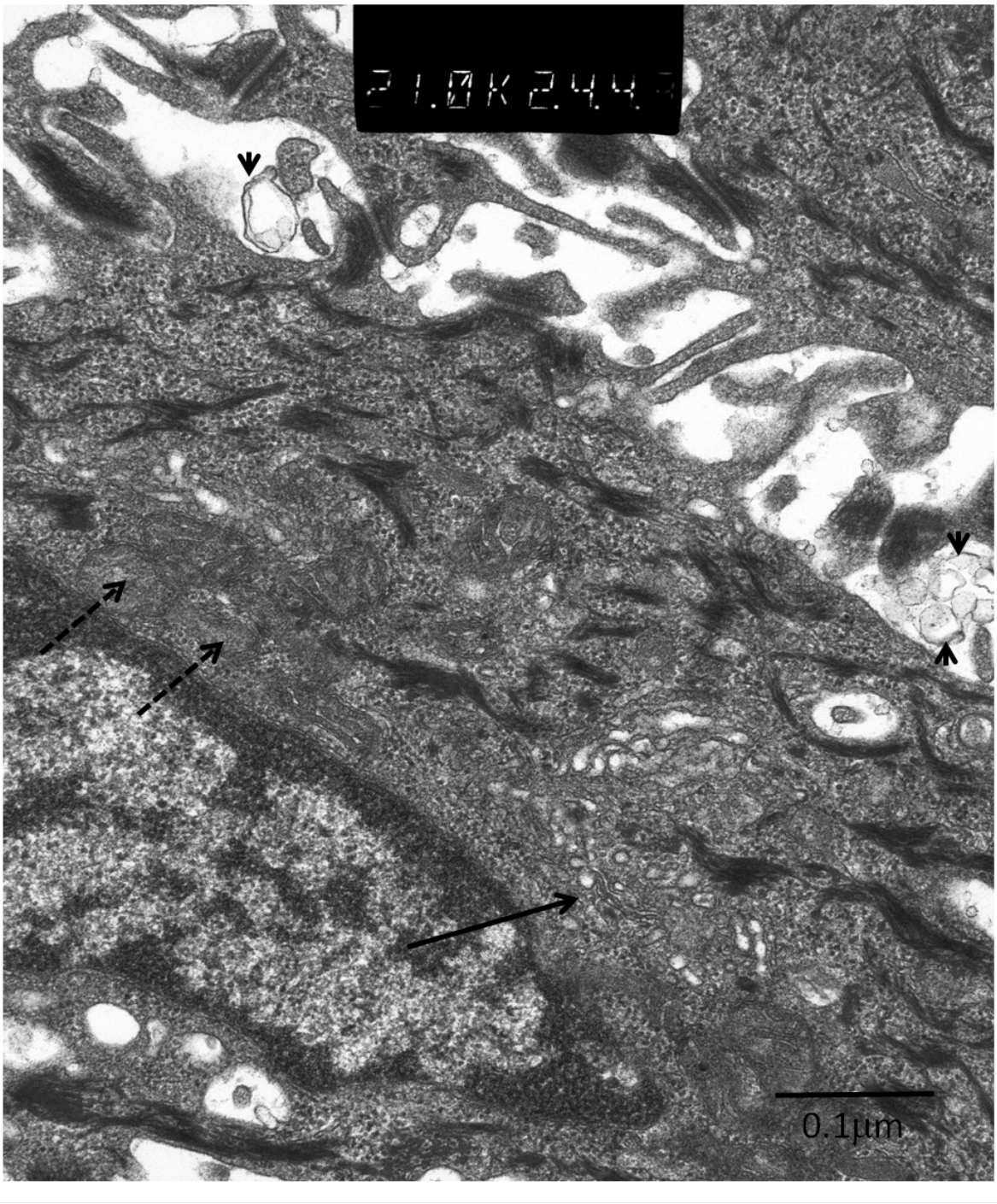
(a) Light microscopy of acral skin (semi-thin section) reveals mild acanthosis, a reduction in the granular layer and compact ortho-hyperkeratosis (original magnification x40; Richardson's stain).



(b) Transmission electron microscopy of basal keratinocytes showing cytoskeletal disruption, numerous mitochondria, and several vesicles close to the plasma membrane.



(c) Transmission electron microscopy of adjacent suprabasal keratinocytes showing cytoskeletal disruption and prominent distended Golgi apparatus in both cells.



(d) Transmission electron microscopy of a keratinocyte within the mid-spinous layer showing cytoskeletal disruption and prominent distended Golgi apparatus and adjacent vesicles (arrow), numerous mitochondria (dashed arrows) and intercellular vesicular collections (arrowheads).

Supplementary Table 1
LOD score calculations for Family 15

Marker	Recombination fraction						
	0	**0.01**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
D15156		0.49	1.02	1.08	0.85	0.49	0.19
D15970	−∞	-5.63	-2.31	-1.13	-0.39	-0.18	-0.07
W1954	−∞	-0.63	0.05	0.26	0.27	0.15	0.05
D15S987	−∞	2.48	2.90	2.78	2.13	1.32	0.54
D15S159	2.73	2.67	2.39	2.03	1.32	0.69	0.25
D15S993	−∞	3.61	3.93	3.73	2.92	1.92	0.89
D15S1018	−∞	-0.19	0.55	0.80	0.82	0.58	0.28
D15S108	1.83	1.81	1.67	1.47	1.03	0.60	0.25
D15S153	−∞	0.63	1.40	1.62	1.49	1.06	0.53
D15S988	3.12	3.12	2.98	2.69	1.90	1.01	0.29
D15S983	8.18	8.04	7.46	6.71	5.11	3.39	1.59
D15S977		1.17	2.18	2.27	1.82	1.13	0.47
Recalculated with probability of misclassification of unaffected family members = 10%							
	Recombination fraction						
	0	0.01	0.05	0.1	0.2	0.3	0.4
D15156	-4.28	0.32	0.85	0.91	0.71	0.40	0.15
D15970	-2.64	-1.79	-0.91	-0.49	-0.21	-0.13	-0.05
W1954	0.52	0.54	0.55	0.52	0.36	0.17	0.05
D15S987	3.25	3.25	3.03	2.73	1.99	1.19	0.47
D15S159	2.45	2.39	2.13	1.79	1.15	0.61	0.22
D15S993	4.15	4.11	3.89	3.53	2.68	1.73	0.79
D15S1018	0.67	0.72	0.83	0.87	0.75	0.50	0.23
D15S108	1.81	1.77	1.61	1.39	0.96	0.55	0.23
D15S153	1.85	1.89	1.93	1.87	1.52	1.02	0.49
D15S988	2.83	2.81	2.65	2.36	1.62	0.84	0.24
D15S983	7.55	7.42	6.87	6.16	4.65	3.05	1.40
D15S977	2.79	2.77	2.65	2.41	1.77	1.05	0.43

Supplementary Table 2

Linkage intervals for PPKP1 on 15q

Flanking markers	**Genomic range (bp, chr 15, GRCh37/hg19)**	**Interval (bp)**	**Reference**
D15S534–D15S818	66,753,604 – 74,230,753	7.48 Mb	5
D15S651–D15S988	66,303,836-67,326,898	1.02 Mb	6
D15S933–D15S977	64,217,749 – 70,454,649	6.24 Mb	This study

Supplementary Table 3

Heterozygous loss-of-function SNPs located on chromosome 15 in PPKP1 Family 1 proband.

Chr15 base number	dbSNP reference	Variation	Gene	Predicted protein mutation
27,777,939	None	T>A	*GABRG3*	p.Leu439Ter
55,722,882	rs57809907	C>A	*DYX1C1*	p.Glu417Ter
67,524,206	None	C>T	*AAGAB*	p.Arg161Ter

Supplementary Table 4

AAGAB mutations identified in this study

DNA mutation	**Protein change**	**Families with this mutation**	**Ancestry**
c.2del	p.0?	1x: Family 16	Scottish
c.140G>A	p.Trp47Ter	2x: Families 9 & 10	Scottish & Irish
c.200_203del	p.Phe67Leufs*41	2x: Families 11 & 12	Japanese
c.344del	p.Asp115Valfs*7	2x: Families 3 & 4	Scottish
c.348_349del	p.Arg116Serfs*1	3x; Families 13-15	Tunisian
c.473del	p.Gly158Glufs*0	5x: Families 3, 5, 6, 8 & 17	Scottish
c.481C>T	p.Arg161Ter	1x: Family 1	Scottish
c.870+1G>A	p.?	2x: Families 7 & 18	Scottish

Supplementary Table 5

PCR primer sequences used in this study.

Primer Name	Strand	Sequence (5'-3')	PCR size
Genomic PCR			
AAGAB1for	+	GGC TAC CTC CGC GGA GG	436 bp
AAGAB1rev	-	AGA AGA ACG CAG GGC CC	
AAGAB2for	+	GCA GGG TCC TTT GAA ATC C	1091 bp
AAGAB4rev	-	TCC TTG AAA CAG CTG GGG	
AAGAB5for1	+	CCA GGC TGG AAT GGT GCC	713 bp
AAGAB5rev1	-	AGC TGG ACT AGG AGC TCC	
AAGAB5for2	+	TGC GCC ACC ACG CCT GGC	451 bp
AAGAB5rev2	-	CGC TTC CTT CAA TCA GCG	
AAGAB6for	+	CTG CAC TCT AGC CTG GGC	376 bp
AAGAB6rev	-	CAT CTA TTT TTA ACA TTC TCT GTC ACC	
AAGAB7for	+	CTA GTT CTT CCT TGG GGG	460 bp
AAGAB7rev	-	GAT AGC CAC TGG CAA GGC	
AAGAB8for	+	AGC TGG TAT ACA CTG GGC C	341 bp
AAGAB8rev	-	GCT GAG ACC AGA ACA AGG	
AAGAB9for	+	CTT TGA AAC CCG TCT CCC	980 bp
AAGAB10rev	-	GGC AGC CAA CAT GAT AAG GGC	
RT-PCR			
AAGAB5intFor	+	CCA AGC CCT GAA TGC C	292 bp
AAGAB8intRev	-	CTC CAC ATC TCC TCC TCC	
cDNA clone			
P34clone1	+	CGG AAT TCC AGC TAT GGC TGC TGG CGT ACC C	940 bp
P34clone2	-	CGA AGC TTT CAG TGC TCT TCA TCA GAT GAA AGG CC	